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Supplementary Material

Functional characterisation of a WRKY transcription factor of wheat and its expression analysis during leaf rust pathogenesis

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Table S1. Summary of Ct valuesTarget and *actin* genes in mock- and pathogen-inoculated wheat NILs at selected time-points

hpi	HD2329				HD2329 + <i>Lr28</i>			
	Mock inoculated		Pathogen inoculated		<i>Mock</i> inoculated		Pathogen inoculated	
	WRKY	<i>Actin</i>	WRKY	<i>Actin</i>	WRKY	<i>Actin</i>	WRKY	<i>Actin</i>
0	36.08±0.248	33.69 ±0.731	34.93±0.351	34.30 ±0.325	35.11 ±0.037	31.41 ±0.311	34.83 ±0.567	33.6 ±0.389
6	33.99±0.378	31.03 ±0.181	35.47±0.284	33.90 ±0.172	34.73 ±0.392	32.15 ±0.801	33.93 ±0.676	32.46 ±0.452
12	32.04±0.162	29.14 ±0.146	31.71±0.398	30.55 ±0.146	34.92 ±0.376	31.46 ±0.612	34.88 ±0.399	33.66 ±0.745
24	30.40±0.11	27.41 ±0.182	32.72±0.277	32.53 ±0.485	38.42±0.094	36.24 ±0.156	30.88 ±0.453	34.37 ±0.51
48	33.78±0.614	30.83 ±0.133	29.92±0.263	29.84 ±0.186	34.42 ±0.239	32.11 ±0.089	32.27 ±0.351	35.57 ±0.182
72	27.87±0.175	24.16 ±0.084	31.17±0.088	29.72 ±0.243	37.34 ±0.288	35.59 ±0.487	31.40±0.367	34.03 ±0.692
168	31.15±0.055	28.01 ±0.481	30.94±0.098	26.39 ±0.423	33.26 ±0.363	31.62 ±0.835	30.16±0.096	32.67 ±0.405

Average Ct values were used for all calculations

Table S2. Docking values of protein and DNA

Parameter	Value
HADDOCK score	-103.2 +/- 6.4
Cluster size	10
RMSD of overall lowest-energy structure	17.9 +/- 0.4
van der Waals energy	-65.7 +/- 4.3
Electrostatic energy	-515.1 +/- 33.0
Desolvation energy	31.0 +/- 2.8
Restraints violation energy	345.1 +/- 17.36
Buried Surface Area	1704.4 +/- 8.2

Table S3. Snapshot of interaction table showing van der Waals interactions at positions within a radius of 3 Å units

112	TRP125A	1030	9230	.310	VDW_INTERACTION
113	ARG126A	104N	9230	.202	VDW_INTERACTION
114	ARG126A	105C	9230	.342	VDW_INTERACTION
115	ARG126A	108C	9210	.700	VDW_INTERACTION
116	ARG126A	108C	936C	.870	VDW_INTERACTION
117	ARG126A	109N	918C	.257	VDW_INTERACTION
118	ARG126A	109N	918C	.381	VDW_INTERACTION
119	ARG126A	110C	9170	.335	VDW_INTERACTION
20	ARG126A	110C	918C	.794	VDW_INTERACTION
21	ARG126A	113X	1512N	.780	VDW_INTERACTION
22	ARG126A	114N	8940	.224	VDW_INTERACTION
23	ARG126A	114N	895C	.400	VDW_INTERACTION
24	ARG126A	114N	918C	.519	VDW_INTERACTION
25	ARG126A	115X	895C	.008	VDW_INTERACTION
26	ARG126A	115X	918C	.884	VDW_INTERACTION
27	ARG126A	116X	8940	.755	VDW_INTERACTION
28	ARG126A	116X	895C	.032	VDW_INTERACTION
29	LYS127A	119N	915C	.406	VDW_INTERACTION
30	LYS127A	119N	916C	.599	VDW_INTERACTION
31	LYS127A	121C	9000	.622	VDW_INTERACTION
32	LYS127A	121C	915C	.627	VDW_INTERACTION
33	LYS127A	123C	9000	.420	VDW_INTERACTION
34	LYS127A	124C	875P	.831	VDW_INTERACTION
35	LYS127A	124C	8760	.297	VDW_INTERACTION
36	LYS127A	124C	879X	.050	VDW_INTERACTION
37	LYS127A	124C	892C	.482	VDW_INTERACTION
38	LYS127A	124C	893C	.847	VDW_INTERACTION
39	LYS127A	124C	896C	.764	VDW_INTERACTION
40	LYS127A	125N	875P	.643	VDW_INTERACTION
41	LYS127A	125N	8760	.317	VDW_INTERACTION
42	LYS127A	125N	8780	.171	VDW_INTERACTION
43	TYR128A	130C	15470	.577	VDW_INTERACTION
44	TYR128A	131C	15460	.661	VDW_INTERACTION
45	TYR128A	132C	1569C	.520	VDW_INTERACTION
46	TYR128A	134C	1556X	.164	VDW_INTERACTION
47	TYR128A	134C	1569C	.407	VDW_INTERACTION
48	TYR128A	138C	879X	.885	VDW_INTERACTION
49	TYR128A	138C	892C	.722	VDW_INTERACTION
50	GLY129A	140N	892C	.496	VDW_INTERACTION
51	GLY129A	141C	875P	.946	VDW_INTERACTION
52	GLY129A	141C	892C	.509	VDW_INTERACTION
53	GLY129A	142C	8760	.441	VDW_INTERACTION
54	GLN130A	148C	8760	.372	VDW_INTERACTION
55	GLN130A	1490	8760	.489	VDW_INTERACTION
56	LYS131A	159C	1580X	.961	VDW_INTERACTION
57	LYS131A	159C	1590C	.573	VDW_INTERACTION
58	LYS131A	160C	15790	.539	VDW_INTERACTION
59	LYS131A	161N	1594C	.259	VDW_INTERACTION
60	LYS131A	161N	1597P	.377	VDW_INTERACTION

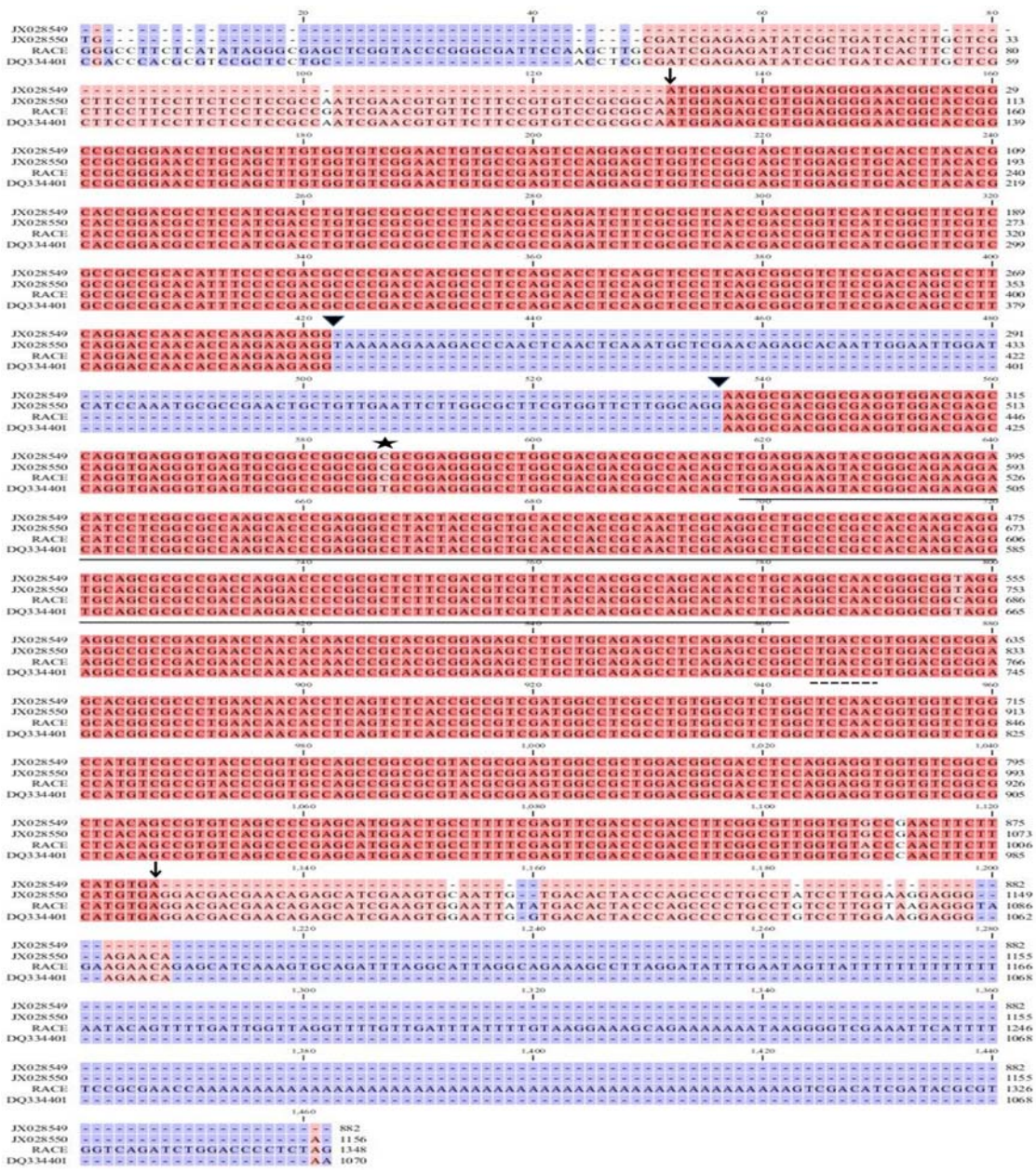
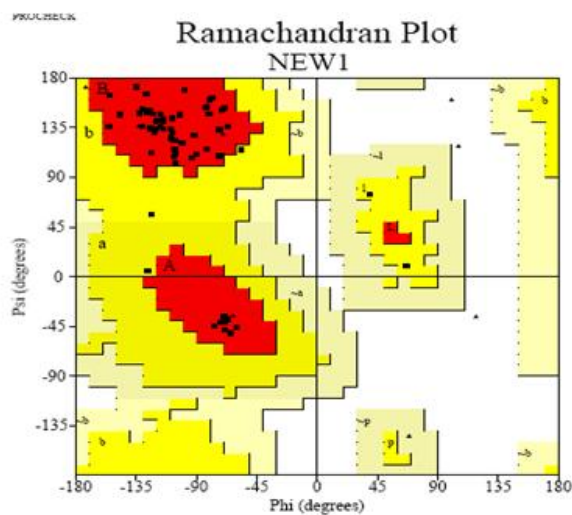


Fig. S1. Aligned nucleotide sequence of the genomic DNA (JX028550), cDNA (JX028549), RACE, and the source sequence (DQ334401). The translation start and stop codons are marked by inverted arrows. The inverted solid triangles represent the intron. The asterisk at position 587 of DQ334401 shows a mutation between the isogenic lines differing by *Lr28*, although *in silico* translation showed the mutation was synonymous. The solid underline represents the sequence supposed to code for the ~60 amino acid DNA-binding domain and the dashed underline correspond to the W-Box element.



Residues in most favoured regions [A,B,L]	54	91.5%
Residues in additional allowed regions [a,b,l,p]	5	8.5%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	0	0.0%
Residues in disallowed regions	0	0.0%

Fig. S2. Ramachandran's plot, obtained through the SAVES server, showing 91.5% of residues in most favored regions and 8.5% in additional allowed regions. No residue occurred in a disallowed region of the plot.

Overall model quality

Z-Score: **-3.04**

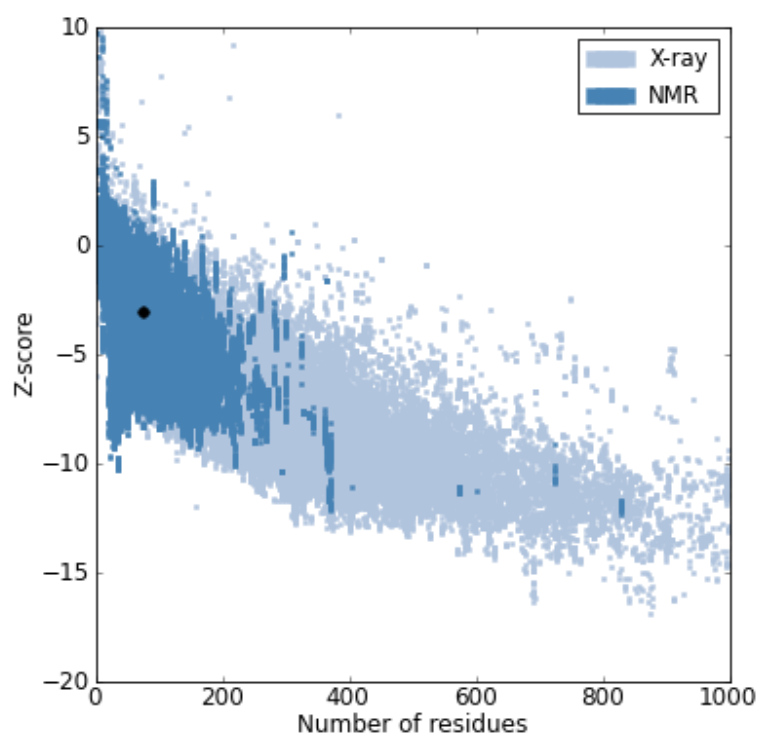


Fig. S3. Overall model quality generated for WRKY JX028549 and obtained through ProSA showing a z-score of -3.04 .

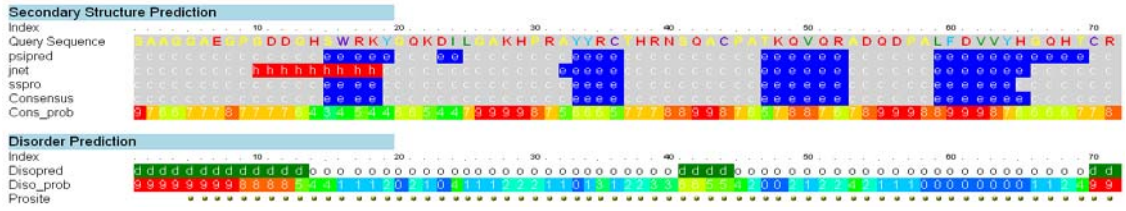


Fig. S4. QuickPhyre alignment of secondary structure prediction indicating four β strands (in blue).

- Highest scoring patch (probable binding site)
- Second highest scoring patch
- Third highest scoring patch

Results

```
SAAGGAEGPG 10 DDGHSWRKYG 20 QK DILGAKHP 30 RAYYRCTHRN 40 SQACPATKQV 50  
QRADQDPALF 60 DWVYHGQHTC 70 RPTG
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Fig. S5. Most appropriate DNA-binding sites within the ~60 amino acid DNA-binding domain predicted by PPI-PRED.

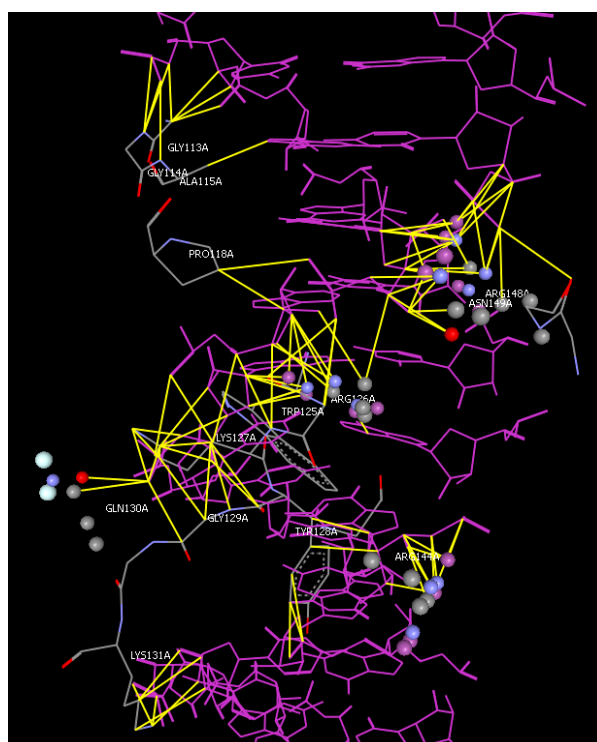


Fig. S6. Van der Waals interactions (in yellow) between the WRKY domain and W-Box within 3 Å of the protein structure and DNA model (in pink).